IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No. 10/664,991 | Customer No. 23379

Applicant: Bjeldanes et al. | Confirmation No. 4613

Filed: Sep 16, 2003 | Group Art Unit: 1614

Docket No. B03-074-1 | Examiner: Betton, Timothy E

Title: *3,3'-Diindolylmethane Antiandrogenic Compositions*

DECLARATION UNDER 37CFR1.131

1. We are the coinventors of this patent application.

2. We invented the claimed subject matter prior to Jun 2003, as documented in the attached:
(i) abstract of our publication "Plant derived 3,3'-diindolylmethane is a strong androgen antagonist in human prostate cancer cells", J Biol Chem 2003 Mar 27 [epub ahead of print], and
(ii) first three pages of our Disclosure and Record of Invention Form, signed Mar 31, 2003 and Apr 7, 2003.

3. Between Mar 27, 2003 and the Sep 16, 2003 filing date of the subject application we were diligent in preparing, reviewing, revising and filing this patent application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application and any patent issuing therefrom.

Date: 9/4/07 — Leonard F. Bjeldanes

Date: 9/7/07 — Gary L. Firestone

Date: 09/07/07 — Hien T. Le

1: J Biol Chem. 2003 Jun 6;278(23):21136-45. Epub 2003 Mar 27.

Plant-derived 3,3'-Diindolylmethane is a strong androgen antagonist in human prostate cancer cells.

Le HT, Schaldach CM, Firestone GL, Bjeldanes LF.

Department of Nutritional Sciences and Toxicology, The University of California, Berkeley, California 94720-3104, USA. lfb@ nature.berkeley.edu

3,3'-Diindolylmethane (DIM) is a major digestive product of indole-3-carbinol, a potential anticancer component of cruciferous vegetables. Our results indicate that DIM exhibits potent antiproliferative and antiandrogenic properties in androgen-dependent human prostate cancer cells. DIM suppresses cell proliferation of LNCaP cells and inhibits dihydrotestosterone (DHT) stimulation of DNA synthesis. These activities were not produced in androgen-independent PC-3 cells. Moreover, DIM inhibited endogenous PSA transcription and reduced intracellular and secreted PSA protein levels induced by DHT in LNCaP cells. Also, DIM inhibited, in a concentration-dependent manner, the DHT-induced expression of a prostate-specific antigen promoter-regulated reporter gene construct in transiently transfected LNCaP cells. Similar effects of DIM were observed in PC-3 cells only when these cells were co-transfected with a wild-type androgen receptor expression plasmid. Using fluorescence imaging with green fluorescent protein androgen receptor and Western blot analysis, we demonstrated that DIM inhibited androgen-induced androgen receptor (AR) translocation into the nucleus. Results of receptor binding assays indicated further that DIM is a strong competitive inhibitor of DHT binding to the AR. Results of structural modeling studies showed that DIM is remarkably similar in conformational geometry and surface charge distribution to an established synthetic AR antagonist, although the atomic compositions of the two substances are quite different. Taken together with our published reports of the estrogen agonist activities of DIM, the present results establish DIM as a unique bifunctional hormone disrupter. To our knowledge, DIM is the first example of a pure androgen receptor antagonist from plants.

PMID: 12665522 [PubMed - indexed for MEDLINE]

Licensing Officer	UNIVERSITY OF CALIFORNIA, BERKELEY OFFICE OF TECHNOLOGY LICENSING **DISCLOSURE AND RECORD OF INVENTION FORM**	B03-074 Case Number
Date Assigned	(please read instructions and complete all pages)	Class Code

Note: When completed, the Disclosure and Record of Invention Form is an important legal document. Care should be taken in its preparation. Please refer to accompanying instructions. If you need assistance, call the Office of Technology Licensing (UC Berkeley Patent Office) at (510) 643-7201. Information contained in this document is maintained in confidence by the the Office of Technology Licensing and normally will not be released to others except with attorney-client privilege, to research sponsors as required by contract, or under appropriate secrecy agreements, until a patent application is filed, the information is published, a determination not to file a patent application is made, or as may be required by law. The information contained should not be disclosed to others outside the University, except as described in section 4(f.), without the approval of the Office of Technology Licensing.

1. Title of Invention:

Indole-3-carbinol and 3,3'-diindolymethane, and derivatives, as antiandrogenic and prostate cancer therapeutic and protective agents.

2. A. Brief Summary of Invention (include novel features and advantages. Use additional sheets if necessary.)

Indole-3-carbinol (I3C) and its derivative, 3,3'-diindolylmethane (DIM), are natural compounds present in cruciferous vegetables. Our continuing studies of the cancer protective effects of these substances have shown that I3C and DIM inhibit the proliferation of androgen sensitive prostate tumor cells by different mechanisms. I3C blocks cell proliferation by a process that involves the selective inhibition of expression of cyclin-dependent kinase 6 (CDK6) protein and transcripts, and stimulated production of the p16 CDK inhibitor protein. DIM, however, can affect prostate tumor cell growth by at least two mechanisms. We have shown that DIM can bind to and block the activity of the androgen receptor (AR), and that DIM can activate the estrogen receptor (ER) by a process that does not involve binding to the receptor. There is considerable evidence in the literature that the combination of AR inhibition and ER activation is of crucial importance in the control of prostate tumorigenesis. Thus, DIM is the first example of a substance that is both a pure AR antagonist and an ER agonist. Because of their multiple antiproliferative mechanisms, the use of I3C and DIM, and more active derivatives, hold great promise for the control of prostate cancer.

B. Detailed Description of Invention (attach additional single-sided sheets)

Identify any references, patent applications, or other publications of which you are aware and which you believe to be pertinent to this invention. Please attach a copy of each of these references, if available.

(see attachment)

3. A. Funding Source/Sponsor — Contract /Grant No.(s) — Principal Investigator

(NOTE: IT IS EXTREMELY IMPORTANT THAT THIS SECTION IS COMPLETED)

Funding Source/Sponsor	Contract /Grant No.(s)	Principal Investigator
~~California Cancer Research Project~~	~~sc#09147V-10010~~	~~PI – L.F. Bjeldanes~~
~~National Institute of Environmental Health Sciences Center~~	~~Grant P30-ES01896~~	~~PI – L.F. Bjeldanes~~

see under disclosure char lit. -Beni

B. This invention utilized data or materials from (check as many as apply):

( ) Celera's proprietary database
( ) Affymetrix chips
( ) A Material Transfer Agreement – "MTA" – (non-UC material)
( ) Other proprietary sources, specify______________).

4. Events

For subject invention, what was the:

	Date	Comments/References
a. Date of first conception of idea	May 1999	
b. Date of first description of complete invention, oral or written conception: identify document, page numbers and location of document	March 2003	
c. Date of first successful demonstration of reduction to practice of invention	Not yet used in practice	
d. Date of first publication containing full description of invention (very important – establishes bar date)	DIM publication in press in JBC – on line 4/1/03 I3C publication in preparation	
e. Dates of external oral disclosures to non-UC employees		
f. Date of planned submission of report, paper, thesis describing invention		

5. If any proprietary material (e.g., cel. , antibody, plasmid, computer software, chemical compound) obtained from outside your laboratory was used to develop this invention under a restrictive written or oral transfer agreement (other than a normal purchasing agreement), please attach a copy or summary of that agreement.

6. INVENTOR INFORMATION. Note: Please fill out completely to allow for timely and accurate distribution of royalty income (to add more inventors go to page 3).

Leonard F. Bjeldanes 3/31/03	3/31/03
Signature Date	Signature Date
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ADD ADDITIONAL INVENTORS ON FOLLOWING PAGES ----------->

7. For any Inventor named (item 6, above) who is not employed full-time by the University of California, please identify other employers (e.g., Veterans Administration, Howard Hughes Medical Institute, USDA), the percent of salary time funded by such other employer, and the nature of the other employment (such as research, teaching or clinical duties).

8. Technically Qualified Witnesses (Two Required) - invention disclosed to and understood by:

3/31/03	3/31/03
Signature Date	Signature Date
JOSEPH L. NAPOLI	SHARON FLEMING
Print name	Print name

Please submit this form with original signatures to:

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Office of Technology Licensing
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INVENTOR INFORMATION (CONTINUED FROM PAGE 2). Note: Please fill out completely to allow for timely and accurate distribution of royalty income.

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